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The Role of Excitatory Amino Acids in Pulsatile Secretion of Luteinizing Hormone in Gilts and Barrows[1,2,3]

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ABSTRACT: The relationship of excitatory amino acid (EAA) activity to LH secretion was investigated in ovariectomized crossbred prepuberal gilts (93 ± 1 kg BW) and Yorkshire barrows (94 ± 2 kg BW) in two experiments. In Exp. 1, eight gilts received, i.m., saline (S) or 20 mg of Ketamine (K)/kg BW, a noncompetitive EAA receptor antagonist. Within these groups, four then received 10 mg of *N*-methyl-DL-aspartate (NMA)/kg BW, an EAA agonist, or S i.v. Mean serum LH concentrations were similar among groups before treatment, did not change after S+S, but decreased ($P < .05$) by 1 h after S+NMA, 3 h after K+S, and 2 h after K+NMA. Serum cortisol concentrations did not change after S+S, but were increased ($P < .05$) from 30 to 90 min after S+NMA, at 120 min after K+S, and from 30 to 120 min after K+NMA. In Exp. 2, barrows received 2.5 mg of NMA/kg BW i.v. immediately after i.m. injection of S (n = 7) or 19.9 mg of K/kg BW (n = 8). Mean serum LH concentrations for the 2 h before treatment were similar among barrows, but decreased ($P < .05$) by 2 h after K+NMA and was not altered after S+NMA. Serum cortisol concentrations were increased at 30 min after S+NMA and from 60 to 90 min after K+NMA. We suggest that EAA both inhibit and stimulate LH secretion, with the inhibitory effects lying within the basal hypothalamus and the stimulatory effects lying within higher brain centers.

Key Words: Pigs, Excitatory Amino Acid, Luteinizing Hormone

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Introduction

Abundant evidence indicates that the excitatory amino acids (**EAA**), L-aspartate and L-glutamate, generally stimulate (via n-methyl-d-aspartate [**NMDA**], kainate, and quisqualate/α-amino-3-hydroxy-5-methylisoxazole-4-propanate receptors) release of neuropeptides from the central nervous system (**CNS**), thereby influencing pituitary function. Sesti and Britt (1992) reported that the specific NMDA agonist, n-methyl-d,l-aspartate (**NMA**), stimulated LH secretion in lactating sows and ovariectomized (**OVX**), estrogen-treated gilts. Estienne et al. (1995) also reported that NMA increased LH secretion in prepuberal gilts. In contrast, Barb et al. (1992) reported that NMA decreased LH secretion in progesterone- and vehicle-treated OVX mature gilts but failed to alter LH secretion in estrogen-treated OVX mature gilts. Chang et al. (1993) also found that NMA suppressed LH secretion in vehicle- and progesterone-treated OVX mature gilts. Because of these conflicting results in mature gilts, an objective was to investigate further the relationship of EAA activity to LH secretion in prepuberal gilts and barrows; the hypothesis was that EAA stimulate LH secretion. In previous studies of Barb et al. (1992) and Chang et al. (1993), NMA stimulated cortisol secretion in gilts. Therefore, a second objective was to confirm this effect of NMA on the pituitary-adrenal axis.

Materials and Methods

Experimental Design. Experiment 1 was conducted at the University of Georgia Swine Center. Sixteen crossbred (Chester × Yorkshire × Hampshire × Duroc)

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[3]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture, the University of Georgia, or the University of Maryland Eastern Shore and does not imply its approval to the exclusion of other products that may be suitable.

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prepuberal gilts, 159 to 161 d of age and 93 ± 1 kg BW, were OVX via midventral laparotomy. All gilts were housed in individual pens in an environmentally controlled building and exposed to a constant temperature of 22°C and an artificial photoperiod of 12:12 h light:dark. Gilts were allowed ad libitum access to water and were fed a corn-soybean meal diet (14% crude protein) fortified with minerals and vitamins according to NRC (1988) guidelines between 0700 and 0800. Two weeks after OVX, four gilts each were assigned to four treatments in a 2 × 2 factorial arrangement. Eight gilts each received i.m. injections of .9% saline (**S**) or 20 mg of ketamine (**K**)/kg BW, a noncompetitive EAA receptor antagonist, which antagonizes excitatory effects mediated by NMDA receptors without affecting responses to quisqualate or kainate receptors (Hansen and Krogsgaard-Larsen, 1990). Within each of these groups, four gilts each then received 10 mg of NMA/kg BW or S i.v., resulting in the following groups: S+NMA, K+NMA, S+S, and K+S. Doses of K and NMA used in the present study were determined previously by Estienne et al. (1996) and Barb et al. (1992), respectively. On the day before treatment, an indwelling catheter was nonsurgically inserted into a jugular vein of each gilt (Barb et al., 1982). Blood samples were collected every 15 min for 2 h before and 8 h after treatment and for an additional 2 h starting 12 h after treatment. All gilts received K or S followed approximately 5 min later by NMA or S. Blood samples were allowed to clot overnight at 4°C, and serum was harvested after centrifugation and stored at −20°C.

Experiment 2 was conducted at the University of Maryland Eastern Shore Swine Research and Education Facility in Princess Anne. Fifteen Yorkshire barrows, 178 d of age and 94 ± 2 kg BW, were housed in individual pens in an environmentally controlled building and exposed to a constant temperature of 23°C and an artificial photoperiod of 12:12 h light: dark. Barrows were allowed ad libitum access to water and feed (16% crude protein, corn-soybean meal diet). Twenty-four hours before administration of treatments, indwelling catheters were nonsurgically inserted into the jugular vein of each barrow. All barrows received 2.5 mg of NMA/kg BW i.v. immediately after i.m. injection of S (n = 7) or 19.9 mg of K/kg BW (n = 8). Doses of NMA and K used were determined in a previous dose-response study of growth hormone (**GH**) secretion, and the blood samples were originally collected for a study of the effects of EAA on GH secretion (Estienne et al., 1996). Blood samples were collected every 15 min for 2 h before and 3 h after treatment and were processed as described in Exp. 1.

Radioimmunoassays. Serum concentrations of LH were quantified in all samples with a RIA described by Kesner et al. (1987). Serum cortisol concentrations were determined for samples collected from all gilts and barrows every 30 min for 2 h before and after treatment with a RIA described by Barb et al. (1992). Assay sensitivities for LH and cortisol were .15 and 1 ng/mL, respectively. Intraassay and interassay CV for LH were 6.3 and 10.3%, respectively. Intraassay and interassay CV for cortisol were 4.0 and 11.8%, respectively. All assays were performed at the Richard B. Russell Agricultural Research Center, USDA-ARS, Athens, GA.

Statistical Analyses. To determine the effect of treatment on serum LH concentration, sampling time was divided into 10 periods for Exp. 1. Period 1 represents the mean of samples collected during the 2 h before treatment and was used to determine whether LH secretion was similar among pigs. Periods 2 through 9 represent the mean of samples collected during each hour following treatment in order to estimate the time course of treatment effects. Period 10 represents the mean of samples collected during the final 2 h, beginning at 12 h after treatment, and was used to determine whether treatment effects were still detectable. In Exp. 2, sampling time was divided into four periods. Period 1 represents the mean of samples collected during the 2 h before treatment, and the remaining time was divided into three 1-h periods. Period means were then subjected to a split plot-in-time ANOVA using the GLM procedure of SAS (1987). The mathematical model included treatment, pig, period, and the treatment × period interaction. Effects of treatment and period were tested using animal within treatment and treatment × period as error terms, respectively. Differences between treatments within period were determined with least squares contrasts (SAS, 1987). For each pig, mean serum LH concentrations, basal serum LH concentrations, number of LH pulses, and LH pulse amplitude were determined from data collected beginning with the sample immediately before injection of NMA and(or) K (not including data from period 10 in Exp. 1) with pulsar analysis using a 5% criterion of variation (Merriam and Watcher, 1982). Data were then subjected to a one-way ANOVA (SAS, 1987). Cortisol data were not divided into hourly periods, because cortisol was assayed in samples collected every 30 min for 2 h before and 2 h after treatment. Data were subjected to a split plot-in-time ANOVA using the GLM procedure of SAS (1987). The mathematical model was the same as that for LH, except sampling time was substituted for period. Differences between sampling times within a treatment were determined with least squares contrasts (SAS, 1987). Comparisons were made with the mean of samples collected at time zero.

Results

Experiment 1. Treatment with K induced immobility and increased salivation, whereas NMA induced vomiting. Mean serum LH concentrations were similar

among groups for the 2 h before injection and averaged .8, .9, .8, and 1.0 ng/mL (pooled SEM = .1) for S+S, S+NMA, K+S, and K+NMA gilts, respectively. Mean serum LH concentrations remained constant after S+S, but they decreased ($P < .05$) after S+NMA, K+S, and K+NMA (Figure 1), resulting in a treatment × time interaction ($P < .001$). The least concentrations were detected by 1 h (.5 ng/mL), 3 h (.5 ng/mL), and 4 h (.3 ng/mL; pooled SEM = .1) after S+NMA, K+S, and K+NMA, respectively (Figure 1). Mean serum LH concentrations were suppressed ($P < .05$) for 2, 3, and 5 h in S+NMA-, K+S-, and K+NMA-treated gilts, respectively (Figure 1). Results of the ANOVA of the data generated by pulsar analysis are presented in Table 1. Mean serum LH concentration, number of LH pulses, and LH pulse amplitude over the 8 h after treatment were not different among treatment groups. However, mean basal serum LH concentration was less ($P < .05$) after K+S and K+NMA than after S+S.

Mean serum cortisol concentrations were similar among groups during the pretreatment period and increased ($P < .05$) after S+NMA, K+S, and K+NMA (Figure 2). Serum cortisol concentrations were increased for 30 to 90 min and 30 to 120 min after S+NMA and K+NMA treatment, respectively, whereas serum cortisol did not increase until 120 min after K+S (Figure 2).

Experiment 2. Behavior was not altered by NMA; however, K induced immobility. Mean serum LH concentrations for the 2 h before treatment (Figure 3) were .8 ± .1 ng/mL for both groups of barrows. Mean serum LH concentrations decreased ($P < .05$) to .5 ± .1 ng/mL by 2 h after K+NMA treatment, whereas serum LH concentration was not altered by S+NMA treatment (.9 ± .1 ng/mL) resulting in a treatment × time interaction ($P < .01$; Figure 3). Results of the ANOVA of the data generated by pulsar analysis are presented in Table 2. Mean serum LH concentration, mean basal serum LH concentration, number of LH pulses, and LH pulse amplitude over the 3 h after treatment were not different among treatment groups. Serum cortisol concentrations were similar among groups during the pretreatment period, but they were increased ($P < .05$) at 30 min after S+NMA and from 60 to 90 min after K+NMA treatment (Figure 4).

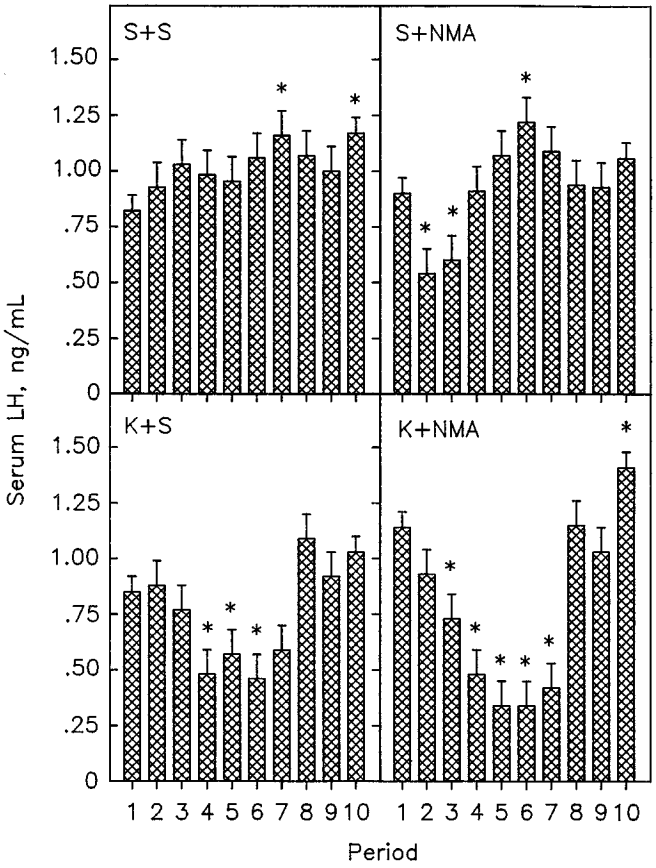

Figure 1. Serum LH concentrations (mean ± SE) before and after the following treatments: saline(S) + S; S + n-methyl-d,l-aspartate (NMA); ketamine (K) + S; or K + NMA in ovariectomized gilts. Period 1 is the 2-h interval before treatment, periods 2 through 9 are hourly intervals after treatment, and period 10 is the 2-h interval starting 12 h after treatment. Within a treatment, bars with an asterisk differ from period 1 ($P < .05$).

Discussion

Results of this study are consistent with previous reports that NMA decreased LH secretion in OVX mature gilts (Barb et al., 1992; Chang et al., 1993)

Table 1. Mean serum luteinizing hormone (LH) concentration (mean ± SE), mean basal serum LH concentration, pulse frequency and amplitude for the 8 h after treatment of gilts with saline (S+S), ketamine + S (K+S), S + n-methyl-d,l-aspartate (S+NMA), and K+NMA

Treatment	No. of gilts	Mean LH, ng/mL	Mean basal LH, ng/mL	No. of LH pulses/8 h	LH pulse amplitude[a], ng/mL
S+S	4	1.0 ± .1	.8 ± .1[b]	7.3 ± .9	.8 ± .2
K+S	4	.7 ± .1	.4 ± .1[c]	5.3 ± .9	1.0 ± .2
S+NMA	4	.9 ± .1	.8 ± .1[b]	4.5 ± .9	1.0 ± .2
K+NMA	4	.7 ± .1	.4 ± .1[c]	4.5 ± .9	1.1 ± .2

[a]Amplitude = LH pulse height – mean basal LH concentration.
[b,c]Within a column, values lacking a common superscript differ ($P < .05$).

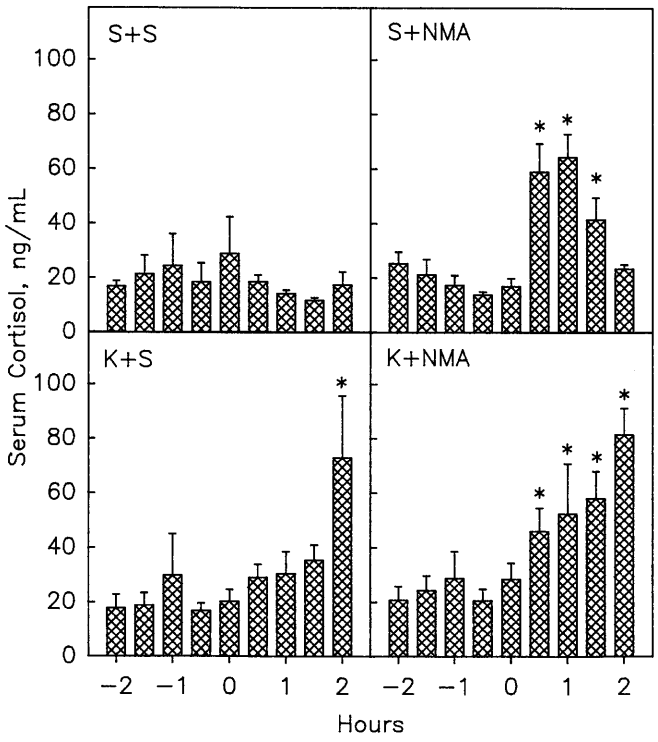

Figure 2. Serum cortisol concentrations (mean ± SE) before and after the following treatments: saline(S) + S; S + n-methyl-d,l-aspartate (NMA); ketamine (K) + S; or K + NMA in ovariectomized gilts. Time of treatment = hour 0. Within a treatment, bars with an asterisk differ from period 1 ($P < .05$).

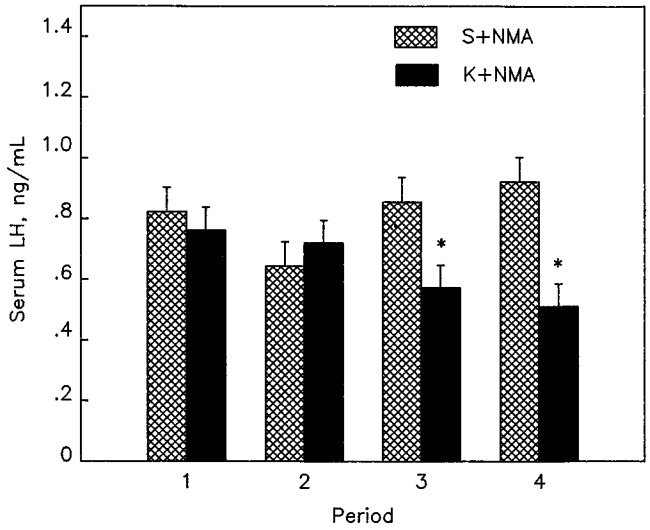

Figure 3. Serum LH concentrations (mean ± SE) for barrows before and after the following treatments: saline (S) + n-methyl-d,l-aspartate (S+NMA); or ketamine (K) + NMA treatment. Period 1 is the 2-h interval before treatment, and periods 2 through 4 are hourly intervals after treatment. Within a treatment, bars with an asterisk differ from period 1 ($P < .05$).

and OVX adult monkeys (Reyes et al., 1990). However, this finding is in contrast to other reports of NMA stimulation of LH secretion in ewes (Estienne et al., 1990), monkeys (Gay and Plant, 1987), rats (Bonavera et al., 1993), and pigs (Sesti and Britt, 1992; Estienne et al., 1995). Sesti and Britt (1992) reported that NMA increased LH secretion in estrogen-treated pigs but had no effect in GnRH antibody-treated gilts. Estrogen may be a necessary antecedent for release of LH after NMA administration in pigs. Replacement of ovarian steroids inhibited NMA-induced suppression of LH secretion in adult OVX rhesus monkeys (Reyes et al., 1991). In our previous study (Barb et al., 1992), NMA had no effect on LH secretion in estrogen-treated gilts, but it decreased serum LH concentrations in OVX and OVX progesterone-treated gilts. Because NMA inhibited LH secretion in mature OVX gilts but not in mature OVX estrogen-treated gilts, only prepuberal OVX gilts and barrows not treated with steroids were used in this study.

Inhibition of LH secretion after i.v. injection of NMA in this study may be due in part to NMA-induced release of other factors that suppress LH secretion. In previous studies, NMA treatment activated the hypothalamic-pituitary-adrenal axis, thus increasing cortisol secretion (Gay and Plant, 1987; Reyes et al., 1991; Barb et al., 1992; Chang et al., 1993). We previously reported that activation of the adrenal axis inhibited pulsatile LH secretion in pigs (Fonda et al., 1984). Reyes et al. (1990) demonstrated that administration of corticotropin-releasing factor (CRF) antiserum to OVX monkeys concomi-

Table 2. Mean serum luteinizing hormone (LH) concentration (mean ± SE), mean basal serum LH concentration, pulse frequency and amplitude for the 3 h after treatment of barrows with saline (S) + n-methyl-d,l-aspartate (S+NMA) or Ketamine (K+NMA)

Treatment	No. of barrows	Mean LH, ng/mL	Mean basal LH, ng/mL	No. of LH pulses/3 h	LH pulse amplitude[a], ng/mL
S+NMA	8	.8 ± .1	.7 ± .1	2.0 ± .3	.5 ± .1
K+NMA	7	.6 ± .1	.5 ± .1	1.2 ± .3	.3 ± .1

[a]Amplitude = pulse height − mean basal LH concentration.

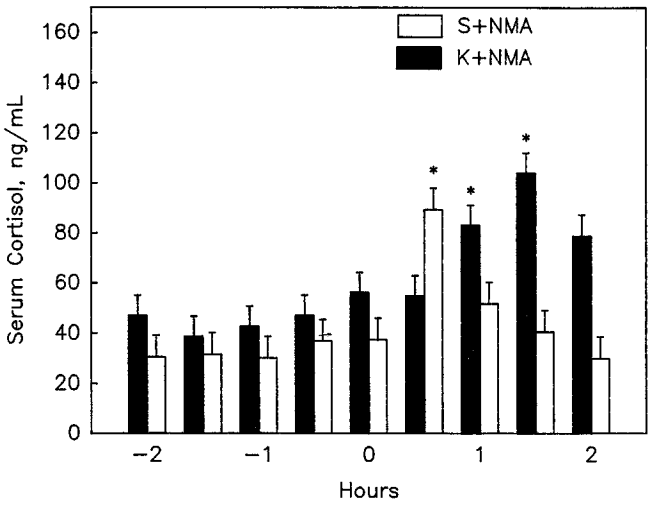

Figure 4. Serum cortisol concentrations (mean ± SE) for barrows before and after the following treatments: saline (S) and n-methyl-d,l-aspartate (NMA); or Ketamine (K) + NMA treatment. Time of treatment = hour 0. Within a treatment, bars with an asterisk differ from period 1 ($P < .05$).

tantly prevented NMA-induced stimulation of cortisol secretion and inhibition of LH secretion and restored normal LH pulse patterns. In addition, administration of the endogenous opioid peptide receptor antagonist naloxone prevented NMA-induced inhibition of LH secretion. Increased cortisol secretion after NMA treatment in gonadectomized animals may be a result of activation of the CRF/β-endorphin pathway (Olster and Ferin, 1987; Reyes et al., 1991), which in turn may act to inhibit GnRH secretion and subsequent LH secretion from the anterior pituitary.

In the present study, inhibition of LH secretion by NMA could have resulted from increased cortisol secretion and release of endogenous opioid peptides (**EOP**). Farah et al. (1989) reported increased secretion of ACTH and β-endorphin after NMA treatment of male rats. Reyes et al. (1990) reported that pretreatment with naloxone, a potent EOP antagonist, prevented NMA-induced decrease in LH secretion. However, in our previous study, Chang et al. (1993) demonstrated that naloxone failed to reverse the NMA-induced decrease in LH secretion in OVX gilts. The apparent dichotomy between those two studies may be due to time of administration relative to NMA and dose of naloxone used. Therefore, EOP modulation of NMA-induced suppression of LH secretion cannot be dismissed.

Although results must be interpreted carefully because of complications arising due to the ability of K to cross the blood-brain barrier, K was used in the present study to determine the specificity of the effects of NMA on LH secretion. Ketamine is the only EAA antagonist of reasonable cost to use in large animals such as pigs. In the present study, serum LH concentrations were suppressed and serum cortisol concentrations were increased longer in K+NMA-treated gilts than in gilts treated with S+NMA or K+S alone. Furthermore, serum cortisol concentrations increased in S+NMA, K+S, and K+NMA gilts before serum LH concentrations decreased. In the present and previous studies (Barb et al., 1992; Chang et al., 1993), NMA increased serum cortisol concentration, which might have acted in synergism with EOP to inhibit LH secretion.

Surprisingly, in the present study, K+NMA treatment decreased serum LH concentrations in gilts and barrows. One would expect K to at least reverse NMA-induced suppression of LH secretion. An explanation for these results is depicted in Figure 5. Because aspartate does not cross the blood-brain barrier (Price et al., 1984), we suggest that NMA may act at the median eminence to inhibit stimulatory mechanisms regulating GnRH secretion and subsequent secretion of LH from the anterior pituitary. However, K crosses the blood-brain barrier and therefore could block endogenous EAA stimulation of GnRH secretion, thereby concealing the ability of K to block the inhibitory effect of NMA at the median eminence. Therefore, the apparent failure of K to reverse NMA-induced inhibition of LH secretion may be due to its activity within the higher CNS (Dripps and Eckenhoff, 1972) to coincidentally inhibit the GnRH pulse generating system and thus inhibit subsequent LH secretion from the pituitary. Therefore, we suggest that mechanisms by which endogenous EAA stimulate LH secretion reside within the blood-brain barrier of the CNS. In support of this idea, Ondo et al. (1988) reported that microinfusion of NMDA into the medial preoptic nucleus of the hypothalamus of rats, where a large number of GnRH perikarya and noradrenergic neurons reside, increased LH secretion from the pituitary. Recently, Lee et al. (1993) reported that intracerebroventricular administration of NMA in diestrous rats increased LH secretion and cFos expression in many regions of the brain, including sites where GnRH perikarya are concentrated. Gay et al. (1993) also reported that intracerebroventricular infusion of NMA in male prepuberal rhesus monkeys provoked GnRH release. However, i.v. administration of the same dose of NMA had no effect on LH release. This may indicate that intracerebroventricular and i.v. administered NMA act differently, either at separate sites or on different populations of GnRH neurons.

Results of these experiments indicate that K+NMA suppressed LH secretion in barrows and OVX gilts, but NMA alone suppressed LH secretion only in OVX gilts. Perhaps the difference in response between OVX gilts and barrows is related in part to differences in sex, nutrition, and(or) dose of NMA used. Because K inhibited LH secretion, EAA may also have stimula-

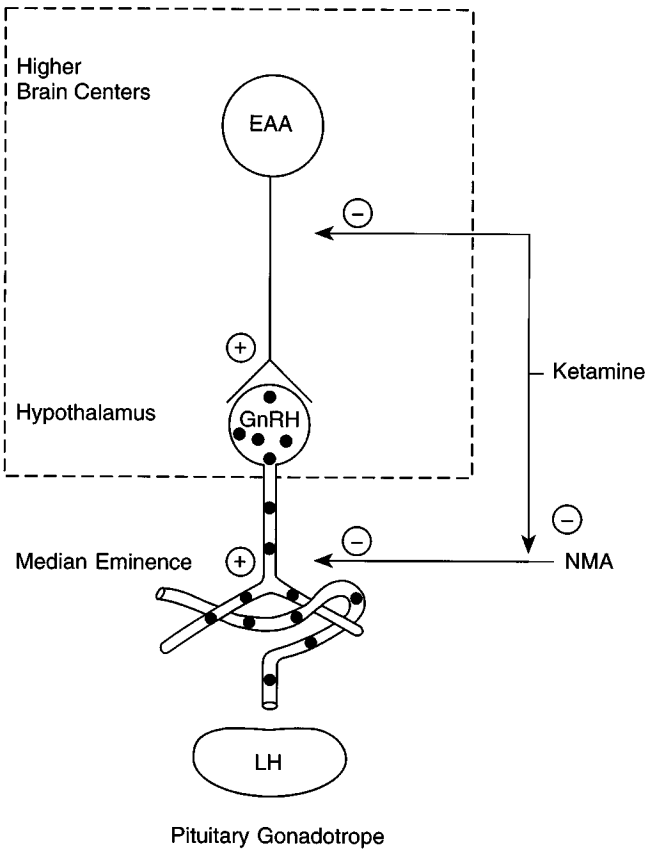

Figure 5. Schematic illustration of Ketamine, n-methyl-d,l-aspartate (NMA), and endogenous excitatory amino acid (EAA) modulation of LH secretion in pigs. - - - Blood-Brain Barrier.

tory effects on pulsatile LH secretion. We suggest that the inhibitory effect lies within the basal hypothalamus, whereas the stimulatory effect lies within the higher brain centers. The inhibitory effect may be due indirectly to activation of the hypothalamo-pituitary-adrenal axis resulting in increased β-endorphin/cortisol secretion, which in turn might feed back negatively on the hypothalamo-pituitary unit to suppress LH secretion.

Implications

Our results indicate that excitatory amino acids, such as aspartate, both inhibit and stimulate luteinizing hormone secretion depending on the site of action within the brain-pituitary unit. Excitatory amino acids may indeed function as endogenous signals that mediate function of gonadotropin-releasing hormone neurons to influence luteinizing hormone secretion, which promotes maturation and ovulation of follicles from the ovary. This mechanism then could be influenced with excitatory amino acid agonists and(or) antagonists to further promote follicular development and increase ovulation rates, thereby increasing reproductive efficiency.

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